STN Search History

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L1    QUE (FAS OR ANTI-FAS) (S) ANTIBOD### (L) METHOTREXATE
L2          44 (FAS OR ANTI-FAS) (S) ANTIBOD### (L) METHOTREXATE

L3          14 DUP REM L2 (30 DUPLICATES REMOVED)

L11         31 (L2 OR L6) AND (AUTOIMMUN#### OR AUTO-IMMUN#### OR RHEUMAT####)


=> d his

     (FILE 'HOME' ENTERED AT 16:08:44 ON 18 MAR 2003)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:09:04 ON
     18 MAR 2003
               SEA (FAS OR ANTI-FAS OR APO-1 OR ?APO-1) (S) ANTIBOD### AND  ME
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              2*  FILE ADISCTI
              0*  FILE ADISINSIGHT
              1*  FILE ADISNEWS
              0*  FILE AGRICOLA
              0*  FILE ANABSTR
              0*  FILE AQUASCI
              0*  FILE BIOBUSINESS
              0*  FILE BIOCOMMERCE
              0*  FILE BIOSIS
              0*  FILE BIOTECHABS
              0*  FILE BIOTECHDS
              0*  FILE BIOTECHNO
              0*  FILE CABA
              0*  FILE CANCERLIT
              0*  FILE CAPLUS
              0*  FILE CEABA-VTB
              0*  FILE CEN
              0*  FILE CIN
              0*  FILE CONFSCI
              0*  FILE CROPB
              0*  FILE CROPU
              0*  FILE DDFB
              4*  FILE DDFU
              0*  FILE DGENE
              0*  FILE DRUGB
              0*  FILE DRUGLAUNCH
              0*  FILE DRUGMONOG2
              0*  FILE DRUGNL
              9*  FILE DRUGU
              0*  FILE DRUGUPDATES
              0*  FILE EMBAL
              0*  FILE EMBASE
              9*  FILE ESBIOBASE
               SEA (FAS OR ANTI-FAS) (S) ANTIBOD###  (L)  METHOTREXATE
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              2   FILE ADISCTI
              1   FILE ADISNEWS
             11   FILE BIOSIS
              7   FILE BIOTECHNO
             10   FILE CANCERLIT
              8   FILE CAPLUS
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                4   FILE DDFU
                8   FILE DRUGU
               12   FILE EMBASE
                9   FILE ESBIOBASE
                0*  FILE FEDRIP
                1   FILE IFIPAT
                3   FILE LIFESCI
               12   FILE MEDLINE
                6   FILE PASCAL
                1   FILE PROMT
               11   FILE SCISEARCH
               13   FILE TOXCENTER
              151   FILE USPATFULL
                3   FILE USPAT2
                1   FILE WPIDS
                1   FILE WPINDEX
L1               QUE (FAS OR ANTI-FAS) (S) ANTIBOD### (L) METHOTREXATE
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     FILE 'MEDLINE, SCISEARCH, BIOSIS, CANCERLIT' ENTERED AT 16:13:23 ON 18
     MAR 2003
L2            44 S (FAS OR ANTI-FAS) (S) ANTIBOD###  (L)  METHOTREXATE
L3            14 DUP REM L2 (30 DUPLICATES REMOVED)
L4            14 S L2 AND SYNERG###
L5             4 DUP REM L4 (10 DUPLICATES REMOVED)
L6           123 S APOPTOSIS AND FAS AND METHOTREXATE
L7             8 S L6 AND SYNERGY
L8            23 S L6 AND SYNERG#####
L9            19 S L8 NOT L5
L10            6 DUP REM L9 (13 DUPLICATES REMOVED)
L11           31 S (L2 OR L6) AND (AUTOIMMUN#### OR AUTO-IMMUN#### OR RHEUMAT##
L12           30 S L11 NOT (L10 OR L5)
L13           14 DUP REM L12 (16 DUPLICATES REMOVED)
L14            3 S L13 AND (METHOTREXATE (S) ANTIBOD###)
L15            4 S L13 AND (METHOTREXATE (L) ANTIBOD###)
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=> d l3 1-14 bib,abs

L3 ANSWER 1 OF 14 MEDLINE DUPLICATE 1
AN 2003010612 IN-PROCESS
DN 22404679 PubMed ID: 12516968
TI Fas-mediated signaling enhances sensitivity of human soft tissue sarcoma cells to anticancer drugs by activation of p38 kinase.
AU Li WeiWei; Bertino Joseph R
CS Program of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.
NC PO1-CA-47179 (NCI)
SO Mol Cancer Ther, (2002 Dec) 1 (14) 1343-8.
Journal code: 101132535. ISSN: 1535-7163.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20030109
Last Updated on STN: 20030109
AB Sensitivity of human soft tissue sarcoma (STS) cells to **methotrexate**, doxorubicin, and paclitaxel was examined after cells were pretreated with CH-11, an agonistic **anti-Fas antibody**. A subtoxic dose (6 ng/ml) of CH-11 sensitized STS cells but not normal fibroblast cells to these anticancer drugs. CH-11 increased cytochrome c release and consequent activation of caspase-9, independent of caspase-8 and increased p38 activation. Addition of SB203580, a specific inhibitor of p38, resulted in a decrease in activation of this kinase and abrogation of enhanced chemosensitivity (doxorubicin and paclitaxel) by CH-11. These results demonstrate that stimulation of the **Fas** pathway by a subtoxic dose of a **Fas** agonist can selectively enhance sensitivity of STS cells to certain chemotherapeutic agents through activation of p38.

L3 ANSWER 2 OF 14 MEDLINE
AN 2002230970 IN-PROCESS
DN 21954176 PubMed ID: 11957195
TI Chronic neutropenia associated with autoimmune disease.
AU Starkebaum Gordon
CS Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.
SO SEMINARS IN HEMATOLOGY, (2002 Apr) 39 (2) 121-7. Ref: 58
Journal code: 0404514. ISSN: 0037-1963.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020424
Last Updated on STN: 20021211
AB Chronic neutropenia with autoimmune diseases is associated mainly with rheumatoid arthritis (RA), as Felty's syndrome or large granular lymphocyte (LGL) leukemia, and with systemic lupus erythematosus (SLE). Recent advances have allowed better understanding regarding the mechanism of neutropenia and improved options for treatment. Target antigens for antineutrophil **antibodies** have been identified for both Felty's syndrome and for SLE. The role of soluble **Fas**-ligand (FasL) in inducing apoptosis of neutrophils has been clarified for LGL leukemia and increased neutrophil apoptosis has been described in neutropenic patients with SLE. The role of immune complexes in affecting neutrophil traffic and function continues to be studied. Treatments of neutropenia have included

methotrexate, cyclosporine A, and granulocyte colony-stimulating factor (G-CSF) as well as granulocyte-macrophage colony-stimulating factor (GM-CSF). The efficacy of both GM- and G-CSF in reversing neutropenia and decreasing the risk of infections in Felty's syndrome and SLE has been well documented. Of concern, however, have been flares of symptoms or development of leukocytoclastic vasculitis in some patients following the use of these cytokines. Recent results suggest that in these patients G-CSF should be administered at the lowest dose effective at elevating the neutrophil count above 1,000/microL.

L3 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:241398 BIOSIS
DN PREV200200241398
TI Immune modulation with low-dose methotrexate involves mitochondrial-mediated apoptosis of CD8+ lymphocytes.
AU Epling-Burnette, P. K. (1); Al-Dawoodie, Nasrin (1); Bai, Fanqui (1); Loughran, Thomas P. (1)
CS (1) Moffitt Cancer Center, IOP, Uni. S. Florida, Tampa, FL USA
SO Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 510a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DT Conference
LA English
AB **Methotrexate** is widely used as a disease-modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA) and other autoimmune diseases. It has also been successfully used for the treatment of Large Granular Lymphocyte (LGL) Leukemia which is characterized by the amplification of CD8+ T lymphocytes with an activated phenotype. Although millions of patients are presently taking low-dose **methotrexate** as immune-modulating therapy, the mechanism of action is poorly understood. We found that doses between 8 nM to 1000 nM selectively induced apoptosis (phosphatidylserine externalization) of the CD8+ lymphocyte population from peripheral blood (PBMC) after activation with PHA and IL-2 and in an acute lymphocytic cell line, CEM. Unactivated PBMC and monocytic cell lines (K562 and HL-60) were resistant to these low doses of **methotrexate**. We found that apoptosis was reversed by pretreatment with folinic acid but not antagonist **anti-Fas antibody** suggesting that inhibition of dihydrofolate reductase (DHFR) is involved in the induction of apoptosis but not ligation of the **Fas** receptor. In addition to externalization of phosphatidylserine (as measured by binding of Annexin-V FITC and detected by flow cytometry), we examined activated PBMC and CEM cells for disruption of mitochondrial membrane potential and DNA fragmentation. We found early evidence of mitochondrial membrane disruption using the Mitotracker(R) (Molecular Probes, Eugene, OR) fluorescent stain and loss of DiOC6 staining by flow cytometry. We also detected the presence of fragmented DNA using a fluorescent tunel assay (Intergen, Purchase, NY). Interestingly, **methotrexate**-induced apoptosis was not reduced by pre-treatment of the cells with a cell permeable caspase 3 inhibitor (Ac-DEVD-fmk, Calbiochem). Also, activation of caspase 3 as indicated by caspase 3 cleavage was not evident by Western blot anlaysis, although readily apparant in cells treated with **anti-Fas** agonistic **antibody** used as a positive control. These data provide us with new and exciting information about the commonly used immune-modulating drug, **methotrexate**. Our data suggests that a proliferating population of lymphocytes are responsive to the apoptotic effects of **methotrexate** after mitochondrial disruption and DNA fragmentation independent of caspase 3 activation. It is important to further delineate the effector caspase (s) or pathways responsible for DNA

fragmentation and cell death given the lymphocyte-specific nature of the apoptotic effects.

L3 ANSWER 4 OF 14 MEDLINE DUPLICATE 2
AN 2000227245 MEDLINE
DN 20227245 PubMed ID: 10766178
TI Anthracyclines trigger apoptosis of both G0-G1 and cycling peripheral blood lymphocytes and induce massive deletion of mature T and B cells.
AU Ferraro C; Quemeneur L; Prigent A F; Taverne C; Revillard J P; Bonnefoy-Berard N
CS Laboratory of Immunology, Institut National de la Sante et de la Recherche Medicale U503 UCBL, Hospital E. Herriot, Lyon, France.
SO CANCER RESEARCH, (2000 Apr 1) 60 (7) 1901-7.
Journal code: 2984705R. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200005
ED Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000504
AB The anthracyclines daunorubicin and doxorubicin were shown to induce apoptosis of hematopoietic cell lines. Here we report that they induce apoptosis of both nonactivated and phytohemagglutinin-activated human peripheral blood lymphocytes. Apoptosis demonstrated by surface expression of phosphatidylserine and typical nuclear alterations reached a maximum after 48 h of incubation with these agents. In contrast to topoisomerase inhibitors (etoposide and camptothecin) and antimetabolites (**methotrexate** and 5-fluorouracil) that induced apoptosis of activated cells only, daunorubicin and doxorubicin triggered apoptosis of cells in the G0-G1 phases of the cell cycle. In agreement with in vitro data, a single i.p. injection of daunorubicin or doxorubicin in BALB/c mice induced T- and B-cell depletion in spleen, lymph nodes, and to a lesser extent in the thymus. Soluble **Fas**-Fc, CD95 antagonistic **antibodies**, as well as the p55 tumor necrosis factor receptor-immunoglobulin fusion protein, did not inhibit drug-induced apoptosis. The level of reactive oxygen species was significantly increased in the presence of daunorubicin or doxorubicin only in nonactivated lymphocytes. However, antioxidants such as N-acetyl-L-cysteine or glutathione did not prevent apoptosis. Activation of caspase-3 after daunorubicin or doxorubicin treatment of either nonactivated or activated lymphocytes was demonstrated by the cleavage of poly(ADP-ribose) polymerase, which was, as apoptosis, inhibited by the peptide benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone. Finally, daunorubicin and doxorubicin induced a rapid production of ceramides. These data indicate that anthracyclines may induce major peripheral T-cell deletion, a property not shared by many cytotoxic agents.

L3 ANSWER 5 OF 14 MEDLINE DUPLICATE 3
AN 2000028377 MEDLINE
DN 20028377 PubMed ID: 10557062
TI Cytotoxic drugs and the CD95 pathway.
AU Friesen C; Fulda S; Debatin K M
CS University Children's Hospital, Ulm; and Division of Molecular Oncology, German Cancer Research Center, Heidelberg, Germany.
SO LEUKEMIA, (1999 Nov) 13 (11) 1854-8. Ref: 57
Journal code: 8704895. ISSN: 0887-6924.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991207
AB Cytotoxic drugs commonly used in chemotherapy of leukemia and solid tumors have been shown to primarily act by inducing apoptosis in sensitive target cells. Apoptosis may involve activation of death-inducing ligand/receptor systems such as CD95 (APO-1/**Fas**). Treatment with anticancer drugs such as doxorubicin, **methotrexate**, cytarabine, etoposide and cisplatin at therapeutic concentrations leads to induction of CD95-ligand (CD95-L). CD95-L can mediate cell death in an autocrine/paracrine manner by crosslinking CD95 receptor (CD95). Interfering with CD95-ligand/receptor interaction by antagonistic **antibodies** to the receptor reduces sensitivity to drug-mediated apoptosis in some cell systems. In addition, treatment with cytotoxic drugs may result in upregulation of CD95, thereby increasing the sensitivity to the CD95 death signal. Apoptosis depends on activation of caspases. Deficient activation of the CD95 system was found in drug-resistant cells. In addition, CD95-resistant and doxorubicin-resistant cells displayed cross-resistance for induction of cell death. Thus, intact apoptosis pathways such as the CD95 system may play a role in determining sensitivity or resistance of tumor cells to chemotherapy.

L3 ANSWER 6 OF 14 MEDLINE DUPLICATE 4
AN 1999304009 MEDLINE
DN 99304009 PubMed ID: 10200578
TI p53-mediated up-regulation of CD95 is not involved in genotoxic drug-induced apoptosis of human breast tumor cells.
AU Ruiz-Ruiz M C; Lopez-Rivas A
CS Instituto de Parasitologia y Biomedicina, CSIC, calle Ventanilla 11, 18001 Granada, Spain.
SO CELL DEATH AND DIFFERENTIATION, (1999 Mar) 6 (3) 271-80.
Journal code: 9437445. ISSN: 1350-9047.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199907
ED Entered STN: 19990806
Last Updated on STN: 19990806
Entered Medline: 19990726
AB Induction of CD95 (**Fas**/APO-1) and CD95 ligand during chemotherapeutic treatment may contribute to the death by apoptosis of some tumor cells. In this study, we have analyzed the role of the CD95 system in genotoxic drug-induced death of human breast tumor cells. Incubation of the breast tumor cell lines MCF-7 and EVSA-T with doxorubicin or **methotrexate** caused apoptosis after 48 h of treatment. These drugs induced a marked increase in the level of CD95 mRNA and protein in wild-type p53-expressing MCF-7 cells. On the contrary, the breast cancer cell line EVSA-T that expresses high levels of an inactive form of p53, did not up-regulate CD95 upon drug treatment. Elevation of CD95 expression by DNA-damaging drugs was notably blocked in MCF-7 cells expressing the human papillomavirus type 16 E6 protein (E6 cells) which prevented p53 accumulation upon DNA damage. However, E6 cells were still killed by the drugs. Furthermore, the genotoxic drugs did not induce the expression of CD95 ligand in MCF-7 cells at doses that caused apoptosis in these breast tumor cells. Moreover, drug-induced apoptosis of breast tumor cells was not prevented in the presence of either a CD95 antagonistic

antibody or a CD95 ligand blocking **antibody**. We also observed a strong synergism between lower doses of DNA-damaging drugs and CD95 agonistic **antibody** in the induction of apoptosis in MCF-7 cells. In summary, our data indicate that drug-induced apoptosis of breast tumor cells occurs by a CD95/CD95L-independent mechanism although by elevating the tumor suppressor proteins p53 and CD95, genotoxic drugs may sensitize breast tumor cells to CD95-mediated apoptosis.

L3 ANSWER 7 OF 14 MEDLINE DUPLICATE 5
AN 1998343523 MEDLINE
DN 98343523 PubMed ID: 9679929
TI Sensitization of human bladder cancer cells to Fas-mediated cytotoxicity by cis-diamminedichloroplatinum (II).
AU Mizutani Y; Yoshida O; Bonavida B
CS Department of Urology, Faculty of Medicine, Kyoto University, Japan.
SO JOURNAL OF UROLOGY, (1998 Aug) 160 (2) 561-70.
Journal code: 0376374. ISSN: 0022-5347.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199808
ED Entered STN: 19980820
Last Updated on STN: 20000303
Entered Medline: 19980813
AB PURPOSE: The resistance of bladder cancer to anticancer chemotherapeutic drugs is a major problem. Several immunotherapeutic approaches have been developed to treat drug-resistant tumor cells. The **Fas** antigen (**Fas**)-**Fas** ligand pathway is involved in cytotoxic T lymphocyte and natural killer cell-mediated cytotoxicity. Like the **Fas** ligand, **anti-Fas** monoclonal **antibody** (mAb) induces apoptosis in tumor cells expressing **Fas**. Several anticancer drugs also mediate apoptosis and may share with **Fas** common intracellular pathways leading to cell killing. We reasoned that treatment of drug-resistant cancer cells with a combination of **anti-Fas** mAb and drugs might overcome their resistance. This study has investigated whether anticancer drugs synergize with **anti-Fas** mAb in cytotoxicity against bladder cancer cells. MATERIALS AND METHODS: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. Synergy was assessed by isobolographic analysis. RESULTS: Treatment of the T24 human bladder cancer cell line with **anti-Fas** mAb in combination with 5-fluorouracil, mitomycin C or **methotrexate** did not overcome resistance to these agents. However, treatment of T24 tumor cells with a combination of **anti-Fas** mAb and cisdiamminedichloroplatinum (II) (CDDP) resulted in a synergistic cytotoxic effect. In addition, the CDDP-resistant T24 line (T24/CDDP) was sensitive to treatment with a combination of **anti-Fas** mAb and CDDP. Synergy by combination of **anti-Fas** mAb and CDDP was also achieved in three other bladder cancer lines and four freshly derived human bladder cancer cells. The combination of **anti-Fas** mAb and carboplatin also resulted in a synergistic cytotoxic effect on T24 cells; however, the combination of **anti-Fas** mAb and trans-diamminedichloroplatinum (II) resulted in an additive cytotoxic effect. Treatment with CDDP enhanced the expression of **Fas** on T24 cells. The synergy achieved in cytotoxicity with **anti-Fas** mAb and CDDP was also achieved in apoptosis. Incubation of T24 cells with **anti-Fas** mAb increased the intracellular accumulation of CDDP. Treatment of freshly isolated bladder cancer cells with CDDP enhanced their susceptibility to lysis by autologous lymphocytes. CONCLUSIONS: This

study demonstrates that combination treatment of bladder cancer cells with **anti-Fas** mAb and CDDP overcomes their resistance. Synergy was achieved with established CDDP-resistant bladder cancer cells and freshly isolated bladder cancer cells. In addition, the sensitization required low concentrations of CDDP, thus supporting the potential in vivo application of combination of CDDP and immunotherapy in the treatment of CDDP- and/or immunotherapy-resistant bladder cancer.

ANSWER 8 OF 14 MEDLINE DUPLICATE 6

AN 1998295656 MEDLINE
DN 98295656 PubMed ID: 9633899
TI Chemotherapeutic drug-induced apoptosis in human leukaemic cells is independent of the Fas (APO-1/CD95) receptor/ligand system.
AU McGahon A J; Costa Pereira A P; Daly L; Cotter T G
CS Department of Biochemistry, University College, Cork, Ireland.
SO BRITISH JOURNAL OF HAEMATOLOGY, (1998 Jun) 101 (3) 539-47.
Journal code: 0372544. ISSN: 0007-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199807
ED Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980731
AB The potential role of the **Fas** (CD95/APO-1) receptor/ligand system in chemotherapeutic drug-induced apoptosis was examined in a number of human leukaemic cell lines. Flow cytometric profiles of doxorubicin-treated HL-60, K562, U937 and Jurkat cells failed to show any significant increase in **Fas** or **Fas** ligand expression over 24 h, despite the induction of significant levels of apoptosis in these cells. Although preincubation of human leukaemic cells with a neutralizing **anti-Fas** IgG **antibody** blocked **anti-Fas** IgM-induced apoptosis, this strategy failed to inhibit chemotherapeutic drug-induced apoptosis. To determine whether recruitment of the **Fas**/**Fas** ligand complex during drug-induced apoptosis was a cell-specific event we utilized the CEM cell line. Doxorubicin treatment of CEM cells over 24 h failed to show any up-regulation in **Fas** or **Fas** ligand protein levels as detected by flow cytometry. Furthermore, neutralizing **anti-Fas** IgG Ab failed to inhibit chemotherapeutic drug-induced apoptosis in CEM cells. The present studies do, however, demonstrate a role for **anti-Fas** IgM Ab in producing a cytotoxic synergistic effect when used in combination with chemotherapeutic drugs. Low-dose **anti-Fas** IgM treatment in combination with doxorubicin, **methotrexate**, camptothecin and etoposide produced an augmented cytoxicity in CEM cells. Taken together these observations demonstrate that although recruitment of the **Fas**/APO-1/CD95 receptor/ligand system is not a necessary requirement for chemotherapeutic drug-induced apoptosis, combination of **anti-Fas** IgM and drug treatment produces a synergistic cytotoxic effect which may prove useful in the treatment of human leukaemias.

L3 ANSWER 9 OF 14 MEDLINE DUPLICATE 7

AN 1998330458 MEDLINE
DN 98330458 PubMed ID: 9664073
TI Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells.
AU Genestier L; Paillot R; Fournel S; Ferraro C; Miossec P; Revillard J P
CS Laboratory of Immunology, Institut National de la Sante et de la Recherche Medicale U80 Claude Bernard University, Hopital E. Herriot, 69437 Lyon, France.

SO JOURNAL OF CLINICAL INVESTIGATION, (1998 Jul 15) 102 (2) 322-8.
Journal code: 7802877. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199808
ED Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980820
AB The folate antagonist **methotrexate** (MTX) is extensively used in graft-versus-host disease, rheumatoid arthritis, and other chronic inflammatory disorders. In addition to its antiinflammatory activity associated with increased release of adenosine, MTX exerts antiproliferative properties by inhibition of dihydrofolate reductase and other folate-dependent enzymes. However, the mechanisms of immunosuppressive properties associated with low-dose MTX treatments are still elusive. We report here that MTX (0.1-10 microM) induces apoptosis of in vitro activated T cells from human peripheral blood. PBL exposed to MTX for 8 h, then activated in drug-free medium, underwent apoptosis, which was completely abrogated by addition of folinic acid or thymidine. Apoptosis of activated T cells did not require interaction between CD95 (**Fas**, APO-1) and its ligand, and adenosine release accounted for only a small part of this MTX activity. Apoptosis required progression of activated T cells to the S phase of the cell cycle, as it was prevented by drugs or **antibodies** that interfere with IL-2 synthesis or signaling pathways. MTX achieved clonal deletion of activated T cells in mixed lymphocyte reactions. Finally, in vitro activation of PBL taken from rheumatoid arthritis patients after MTX injection resulted in apoptosis. Altogether, the data demonstrate that MTX can selectively delete activated peripheral blood T cells by a CD95-independent pathway. This property could be used as a new pharmacological end point to optimize dosage and timing of MTX administration. It may account for the immunosuppressive effects of low-dose MTX treatments.

L3 ANSWER 10 OF 14 MEDLINE DUPLICATE 8
AN 97223915 MEDLINE
DN 97223915 PubMed ID: 9070496
TI Doxorubicin sensitizes human bladder carcinoma cells to Fas-mediated cytotoxicity.
AU Mizutani Y; Okada Y; Yoshida O; Fukumoto M; Bonavida B
CS Department of Urology, Faculty of Medicine, Kyoto University, Japan.
SO CANCER, (1997 Mar 15) 79 (6) 1180-9.
Journal code: 0374236. ISSN: 0008-543X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199704
ED Entered STN: 19970424
Last Updated on STN: 19970424
Entered Medline: 19970415
AB BACKGROUND: The resistance of bladder carcinoma to anticancer chemotherapeutic agents remains a major problem. Hence, several immunotherapeutic approaches have been developed to treat the drug-resistant cancer cells. **Fas** antigen (**Fas**) and **Fas** ligand participate in cytotoxicity mediated by T lymphocytes and natural killer cells. Like **Fas** ligand, **anti-Fas** monoclonal **antibody** (MoAb) induces apoptosis of the cells expressing **Fas**. This study examined whether bladder carcinoma cells are sensitive to cytotoxicity mediated by **anti-**

Fas MoAb and whether anticancer agents synergize with **anti-Fas** MoAb in cytotoxicity. METHODS: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. Synergy was assessed by isobolographic analysis. RESULTS: The T24 human bladder carcinoma cell line constitutively expressed the **Fas** on the cell surface; however, T24 line was resistant to **anti-Fas** MoAb. Treatment of T24 cells with **anti-Fas** MoAb in combination with mitomycin C, **methotrexate**, or 5-fluorouracil did not overcome their resistance to these agents. However, treatment of T24 cells with a combination of **anti-Fas** MoAb and doxorubicin resulted in a synergistic cytotoxic effect. In addition, the doxorubicin-resistant T24 cells were sensitive to treatment with a combination of **anti-Fas** MoAb and doxorubicin. Synergy was also achieved in three other bladder carcinoma cell lines and four freshly derived human bladder carcinoma cells. Treatment with **anti-Fas** MoAb in combination with epirubicin or pirarubicin also resulted in a synergistic cytotoxic effect on T24 cells. The mechanisms of synergy were examined. **Anti-Fas** MoAb did not affect the intracellular accumulation of doxorubicin, the expression of P-glycoprotein, or the expression of the antioxidant glutathione S-transferase-pi mRNA. However, treatment with doxorubicin enhanced the expression of **Fas** on T24 cells. CONCLUSIONS: This study demonstrated that treatment of bladder carcinoma cells with doxorubicin sensitized the cells to lysis by **anti-Fas** MoAb. The synergistic effect obtained with established doxorubicin-resistant bladder carcinoma cells and freshly isolated bladder carcinoma cells suggests that drug-resistant bladder carcinoma cells can be sensitized by doxorubicin to **Fas**- and **Fas** ligant-mediated cytotoxicity by lymphocytes. Furthermore, the sensitization required low concentrations of doxorubicin, thus supporting the in vivo application of a combination of chemotherapy and immunotherapy in the treatment of drug-resistant and/or immunotherapy-resistant bladder carcinoma.

L3 ANSWER 12 OF 14 CANCERLIT
AN 97621016 CANCERLIT
DN 97621016
TI Evidence for the involvement of the Fas pathway in methotrexate-induced cell death (Meeting abstract).
AU Bhushan A; Stone J E; Hacker M P; Tritton T R; Newell K
CS M Vermont Cancer Center, Departments of Medicine and Pharmacology, University of Vermont, Burlington, VT.
SO Proc Annu Meet Am Assoc Cancer Res, (1997) 38 A609.
ISSN: 0197-016X.
DT (MEETING ABSTRACTS)
LA English
FS Institute for Cell and Developmental Biology
EM 199710
ED Entered STN: 19980417
Last Updated on STN: 19980417
AB L1210/DDP is a **methotrexate** resistant line derived by selection of L1210 leukemia cells with cisplatin. Our previous work indicates that normal B cells expressing low levels of MHC class II molecules die by apoptosis when MHC class II is ligated and the mechanism of death involves **Fas**. Increased expression of **Fas** and B7-1 and B7-2 have been shown to result from MHC class II engagement. Here we address the possibility that the drug resistant phenotype may be characterized by changes in MHC class II, B7-1, B7-2 or **Fas**. We measured the cell surface expression of MHC class II, B7-1, B7-2, and **Fas** on L1210 and L1210/DDP cells by flow cytometry. L1210 cells express intermediate levels of MHC class II and B7-1, high levels of **Fas**, and no B7-2. L1210 DDP cells express high levels of MHC class II and B7-2,

moderate levels of B7-1, and low levels of **Fas**. We reasoned that changes in the cell surface expression of these molecules on the L1210DDP cells may be a reflection of alterations in MHC class II mediated, **Fas** dependent cell death. Pretreatment of the cells with **antibody** to MHC class II, followed by culturing overnight on **anti-Fas** coated plates resulted in death of only the L1210 cells. This is consistent with the hypothesis that drug resistance is related to **Fas** resistance and support the possibility that the cytotoxic effect of some anti-cancer drugs may involve the **Fas** pathway.

L3 ANSWER 13 OF 14 MEDLINE DUPLICATE 10
AN 97174339 MEDLINE
DN 97174339 PubMed ID: 9022073
TI Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53.
AU Muller M; Strand S; Hug H; Heinemann E M; Walczak H; Hofmann W J; Stremmel W; Krammer P H; Galle P R
CS University Hospital, Department of Internal Medicine IV, Heidelberg, Germany.
SO JOURNAL OF CLINICAL INVESTIGATION, (1997 Feb 1) 99 (3) 403-13.
Journal code: 7802877. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199703
ED Entered STN: 19970321
Last Updated on STN: 19970321
Entered Medline: 19970310
AB Chemotherapeutic drugs are cytotoxic by induction of apoptosis in drug-sensitive cells. We investigated the mechanism of bleomycin-induced cytotoxicity in hepatoma cells. At concentrations present in the sera of patients during therapy, bleomycin induced transient accumulation of nuclear wild-type (wt) p53 and upregulated expression of cell surface CD95 (APO-1/**Fas**) receptor in hepatoma cells carrying wt p53 (HepG2). Bleomycin did not increase CD95 in hepatoma cells with mutated p53 (Huh7) or in hepatoma cells which were p53-/- (Hep3B). In addition, sensitivity towards CD95-mediated apoptosis was also increased in wt p53 positive HepG2 cells. Microinjection of wt p53 cDNA into HepG2 cells had the same effect. In contrast, bleomycin did not enhance susceptibility towards CD95-mediated apoptosis in Huh7 and in Hep3B cells. Furthermore, bleomycin treatment of HepG2 cells increased CD95 ligand (CD95L) mRNA expression. Most notably, bleomycin-induced apoptosis in HepG2 cells was almost completely inhibited by **antibodies** which interfere with CD95 receptor/ligand interaction. These data suggest that apoptosis induced by bleomycin is mediated, at least in part, by p53-dependent stimulation of the CD95 receptor/ligand system. The same applies to other anti-cancer drugs such as cisplatin and **methotrexate**. These data may have major consequences for drug treatment of cancer and the explanation of drug sensitivity and resistance.

L3 ANSWER 14 OF 14 MEDLINE DUPLICATE 11
AN 96206283 MEDLINE
DN 96206283 PubMed ID: 8616718
TI Involvement of the CD95 (APO-1/FAS) receptor/ligand system in drug-induced apoptosis in leukemia cells.
AU Friesen C; Herr I; Krammer P H; Debatin K M
CS Department of Hematology/Oncology, University Children's Hospital, Heidelberg, Germany.

SO NATURE MEDICINE, (1996 May) 2 (5) 574-7.
Journal code: 9502015. ISSN: 1078-8956.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199606
ED Entered STN: 19960620
Last Updated on STN: 19970203
Entered Medline: 19960613
AB Cytotoxic drugs used in chemotherapy of leukemias and solid tumors cause apoptosis in target cells. In lymphoid cells the CD95 (APO-1/**Fas**)/CD95 ligand (CD95-L) system is a key regulator of apoptosis. Here we describe that doxorbicin induces apoptosis via the CD95/CD95-L system in human leukemia T-cell lines. Doxorubicin-induced apoptosis was completely blocked by inhibition of gene expression and protein synthesis. Also, doxorbicin strongly stimulates CD95-L messenger RNA expression in vitro at concentrations relevant for therapy in vivo. CEM and jurkat cells resistant to CD95-mediated apoptosis were also resistant to doxorbicin-induced apoptosis. Furthermore, doxorbicin-induced apoptosis was inhibited by blocking F(ab')2 anti-APO-1 (anti-CD95) **antibody** fragments. Expression of CD95-L mRNA and protein in vitro was also stimulated by other cytotoxic drugs such as **methotrexate**. The finding that apoptosis caused by anticancer drugs may be mediated via the CD95 system provides a new molecular insight into resistance and sensitivity toward chemotherapy in malignancies.

L5 ANSWER 1 OF 4 MEDLINE DUPLICATE 1
AN 1999304009 MEDLINE
DN 99304009 PubMed ID: 10200578
TI p53-mediated up-regulation of CD95 is not involved in genotoxic drug-induced apoptosis of human breast tumor cells.
AU Ruiz-Ruiz M C; Lopez-Rivas A
CS Instituto de Parasitologia y Biomedicina, CSIC, calle Ventanilla 11, 18001 Granada, Spain.
SO CELL DEATH AND DIFFERENTIATION, (1999 Mar) 6 (3) 271-80.
Journal code: 9437445. ISSN: 1350-9047.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199907
ED Entered STN: 19990806
Last Updated on STN: 19990806
Entered Medline: 19990726
AB Induction of CD95 (**Fas**/APO-1) and CD95 ligand during chemotherapeutic treatment may contribute to the death by apoptosis of some tumor cells. In this study,. . . drug-induced death of human breast tumor cells. Incubation of the breast tumor cell lines MCF-7 and EVSA-T with doxorubicin or **methotrexate** caused apoptosis after 48 h of treatment. These drugs induced a marked increase in the level of CD95 mRNA and. . . tumor cells. Moreover, drug-induced apoptosis of breast tumor cells was not prevented in the presence of either a CD95 antagonistic **antibody** or a CD95 ligand blocking **antibody** . We also observed a strong **synergism** between lower doses of DNA-damaging drugs and CD95 agonistic **antibody** in the induction of apoptosis in MCF-7 cells. In summary, our data indicate that drug-induced apoptosis of breast tumor cells. . .

L5 ANSWER 2 OF 4 MEDLINE DUPLICATE 2
AN 1998343523 MEDLINE
DN 98343523 PubMed ID: 9679929
TI Sensitization of human bladder cancer cells to Fas-mediated cytotoxicity by cis-diamminedichloroplatinum (II).
AU Mizutani Y; Yoshida O; Bonavida B
CS Department of Urology, Faculty of Medicine, Kyoto University, Japan.
SO JOURNAL OF UROLOGY, (1998 Aug) 160 (2) 561-70.
Journal code: 0376374. ISSN: 0022-5347.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199808
ED Entered STN: 19980820
Last Updated on STN: 20000303
Entered Medline: 19980813
AB . . . to anticancer chemotherapeutic drugs is a major problem. Several immunotherapeutic approaches have been developed to treat drug-resistant tumor cells. The **Fas** antigen (**Fas**)-**Fas** ligand pathway is involved in cytotoxic T lymphocyte and natural killer cell-mediated cytotoxicity. Like the **Fas** ligand, **anti-Fas** monoclonal **antibody** (mAb) induces apoptosis in tumor cells expressing **Fas**. Several anticancer drugs also mediate apoptosis and may share with **Fas** common intracellular pathways leading to cell killing. We reasoned that treatment of drug-resistant cancer cells with a combination of **anti-Fas** mAb and drugs might overcome their resistance. This study has investigated whether anticancer drugs **synergize** with **anti-Fas** mAb

in cytotoxicity against bladder cancer cells. MATERIALS AND METHODS: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. **Synergy** was assessed by isobolographic analysis. RESULTS: Treatment of the T24 human bladder cancer cell line with **anti-Fas** mAb in combination with 5-fluorouracil, mitomycin C or **methotrexate** did not overcome resistance to these agents. However, treatment of T24 tumor cells with a combination of **anti-Fas** mAb and cisdiamminedichloroplatinum (II) (CDDP) resulted in a synergistic cytotoxic effect. In addition, the CDDP-resistant T24 line (T24/CDDP) was sensitive to treatment with a combination of **anti-Fas** mAb and CDDP. **Synergy** by combination of **anti-Fas** mAb and CDDP was also achieved in three other bladder cancer lines and four freshly derived human bladder cancer cells. The combination of **anti-Fas** mAb and carboplatin also resulted in a synergistic cytotoxic effect on T24 cells; however, the combination of **anti-Fas** mAb and trans-diamminedichloroplatinum (II) resulted in an additive cytotoxic effect. Treatment with CDDP enhanced the expression of **Fas** on T24 cells. The **synergy** achieved in cytotoxicity with **anti-Fas** mAb and CDDP was also achieved in apoptosis. Incubation of T24 cells with **anti-Fas** mAb increased the intracellular accumulation of CDDP. Treatment of freshly isolated bladder cancer cells with CDDP enhanced their susceptibility to lysis by autologous lymphocytes. CONCLUSIONS: This study demonstrates that combination treatment of bladder cancer cells with **anti-Fas** mAb and CDDP overcomes their resistance. **Synergy** was achieved with established CDDP-resistant bladder cancer cells and freshly isolated bladder cancer cells. In addition, the sensitization required low. . .

L5 ANSWER 3 OF 4 MEDLINE DUPLICATE 3
AN 1998295656 MEDLINE
DN 98295656 PubMed ID: 9633899
TI Chemotherapeutic drug-induced apoptosis in human leukaemic cells is independent of the Fas (APO-1/CD95) receptor/ligand system.
AU McGahon A J; Costa Pereira A P; Daly L; Cotter T G
CS Department of Biochemistry, University College, Cork, Ireland.
SO BRITISH JOURNAL OF HAEMATOLOGY, (1998 Jun) 101 (3) 539-47.
Journal code: 0372544. ISSN: 0007-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199807
ED Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980731
AB The potential role of the **Fas** (CD95/APO-1) receptor/ligand system in chemotherapeutic drug-induced apoptosis was examined in a number of human leukaemic cell lines. Flow cytometric profiles of doxorubicin-treated HL-60, K562, U937 and Jurkat cells failed to show any significant increase in **Fas** or **Fas** ligand expression over 24 h, despite the induction of significant levels of apoptosis in these cells. Although preincubation of human leukaemic cells with a neutralizing **anti-Fas** IgG **antibody** blocked **anti-Fas** IgM-induced apoptosis, this strategy failed to inhibit chemotherapeutic drug-induced apoptosis. To determine whether recruitment of the **Fas**/**Fas** ligand complex during drug-induced apoptosis was a cell-specific event we utilized the CEM cell line. Doxorubicin treatment of CEM cells over 24 h failed to show any up-regulation in **Fas** or **Fas** ligand protein levels as detected by flow cytometry. Furthermore, neutralizing **anti-**

Fas IgG Ab failed to inhibit chemotherapeutic drug-induced apoptosis in CEM cells. The present studies do, however, demonstrate a role for **anti-Fas** IgM Ab in producing a cytotoxic synergistic effect when used in combination with chemotherapeutic drugs. Low-dose **anti-Fas** IgM treatment in combination with doxorubicin, **methotrexate**, camptothecin and etoposide produced an augmented cytoxicity in CEM cells. Taken together these observations demonstrate that although recruitment of the **Fas**/APO-1/CD95 receptor/ligand system is not a necessary requirement for chemotherapeutic drug-induced apoptosis, combination of **anti-Fas** IgM and drug treatment produces a synergistic cytotoxic effect which may prove useful in the treatment of human leukaemias.

L5 ANSWER 4 OF 4 MEDLINE DUPLICATE 4
AN 97223915 MEDLINE
DN 97223915 PubMed ID: 9070496
TI Doxorubicin sensitizes human bladder carcinoma cells to Fas-mediated cytotoxicity.
AU Mizutani Y; Okada Y; Yoshida O; Fukumoto M; Bonavida B
CS Department of Urology, Faculty of Medicine, Kyoto University, Japan.
SO CANCER, (1997 Mar 15) 79 (6) 1180-9.
Journal code: 0374236. ISSN: 0008-543X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199704
ED Entered STN: 19970424
Last Updated on STN: 19970424
Entered Medline: 19970415
AB . . . anticancer chemotherapeutic agents remains a major problem. Hence, several immunotherapeutic approaches have been developed to treat the drug-resistant cancer cells. **Fas** antigen (**Fas**) and **Fas** ligand participate in cytotoxicity mediated by T lymphocytes and natural killer cells. Like **Fas** ligand, **anti-Fas** monoclonal **antibody** (MoAb) induces apoptosis of the cells expressing **Fas**. This study examined whether bladder carcinoma cells are sensitive to cytotoxicity mediated by **anti-Fas** MoAb and whether anticancer agents **synergize** with **anti-Fas** MoAb in cytotoxicity. METHODS: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. **Synergy** was assessed by isobolographic analysis. RESULTS: The T24 human bladder carcinoma cell line constitutively expressed the **Fas** on the cell surface; however, T24 line was resistant to **anti-Fas** MoAb. Treatment of T24 cells with **anti-Fas** MoAb in combination with mitomycin C, **methotrexate**, or 5-fluorouracil did not overcome their resistance to these agents. However, treatment of T24 cells with a combination of **anti-Fas** MoAb and doxorubicin resulted in a synergistic cytotoxic effect. In addition, the doxorubicin-resistant T24 cells were sensitive to treatment with a combination of **anti-Fas** MoAb and doxorubicin. **Synergy** was also achieved in three other bladder carcinoma cell lines and four freshly derived human bladder carcinoma cells. Treatment with **anti-Fas** MoAb in combination with epirubicin or pirarubicin also resulted in a synergistic cytotoxic effect on T24 cells. The mechanisms of **synergy** were examined. **Anti-Fas** MoAb did not affect the intracellular accumulation of doxorubicin, the expression of P-glycoprotein, or the expression of the antioxidant glutathione S-transferase-pi mRNA. However, treatment with doxorubicin enhanced the expression of **Fas** on T24 cells. CONCLUSIONS: This study demonstrated that treatment of bladder carcinoma cells with doxorubicin sensitized the cells to lysis by **anti-**

Fas MoAb. The synergistic effect obtained with established doxorubicin-resistant bladder carcinoma cells and freshly isolated bladder carcinoma cells suggests that drug-resistant bladder carcinoma cells can be sensitized by doxorubicin to **Fas**- and **Fas** ligant-mediated cytotoxicity by lymphocytes. Furthermore, the sensitization required low concentrations of doxorubicin, thus supporting the in vivo application of a. . .

CT . . .

L5 ANSWER 1 OF 4 MEDLINE DUPLICATE 1
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SO CELL DEATH AND DIFFERENTIATION, (1999 Mar) 6 (3) 271-80.
Journal code: 9437445. ISSN: 1350-9047.
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EM 199907
ED Entered STN: 19990806
Last Updated on STN: 19990806
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AB Induction of CD95 (**Fas**/APO-1) and CD95 ligand during chemotherapeutic treatment may contribute to the death by apoptosis of some tumor cells. In this study,. . . drug-induced death of human breast tumor cells. Incubation of the breast tumor cell lines MCF-7 and EVSA-T with doxorubicin or **methotrexate** caused apoptosis after 48 h of treatment. These drugs induced a marked increase in the level of CD95 mRNA and. . . tumor cells. Moreover, drug-induced apoptosis of breast tumor cells was not prevented in the presence of either a CD95 antagonistic **antibody** or a CD95 ligand blocking **antibody** . We also observed a strong **synergism** between lower doses of DNA-damaging drugs and CD95 agonistic **antibody** in the induction of apoptosis in MCF-7 cells. In summary, our data indicate that drug-induced apoptosis of breast tumor cells. . .

L5 ANSWER 2 OF 4 MEDLINE DUPLICATE 2
AN 1998343523 MEDLINE
DN 98343523 PubMed ID: 9679929
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AU Mizutani Y; Yoshida O; Bonavida B
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EM 199808
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L5 ANSWER 3 OF 4 MEDLINE DUPLICATE 3
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EM 199807
ED Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980731
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AU Mizutani Y; Okada Y; Yoshida O; Fukumoto M; Bonavida B
CS Department of Urology, Faculty of Medicine, Kyoto University, Japan.
SO CANCER, (1997 Mar 15) 79 (6) 1180-9.
Journal code: 0374236. ISSN: 0008-543X.
CY United States
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LA English
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EM 199704
ED Entered STN: 19970424
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Entered Medline: 19970415
AB . . . anticancer chemotherapeutic agents remains a major problem. Hence, several immunotherapeutic approaches have been developed to treat the drug-resistant cancer cells. **Fas** antigen (**Fas**) and **Fas** ligand participate in cytotoxicity mediated by T lymphocytes and natural killer cells. Like **Fas** ligand, **anti-Fas** monoclonal **antibody** (MoAb) induces apoptosis of the cells expressing **Fas**. This study examined whether bladder carcinoma cells are sensitive to cytotoxicity mediated by **anti-Fas** MoAb and whether anticancer agents **synergize** with **anti-Fas** MoAb in cytotoxicity. METHODS: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. **Synergy** was assessed by isobolographic analysis. RESULTS: The T24 human bladder carcinoma cell line constitutively expressed the **Fas** on the cell surface; however, T24 line was resistant to **anti-Fas** MoAb. Treatment of T24 cells with **anti-Fas** MoAb in combination with mitomycin C, **methotrexate**, or 5-fluorouracil did not overcome their resistance to these agents. However, treatment of T24 cells with a combination of **anti-Fas** MoAb and doxorubicin resulted in a synergistic cytotoxic effect. In addition, the doxorubicin-resistant T24 cells were sensitive to treatment with a combination of **anti-Fas** MoAb and doxorubicin. **Synergy** was also achieved in three other bladder carcinoma cell lines and four freshly derived human bladder carcinoma cells. Treatment with **anti-Fas** MoAb in combination with epirubicin or pirarubicin also resulted in a synergistic cytotoxic effect on T24 cells. The mechanisms of **synergy** were examined. **Anti-Fas** MoAb did not affect the intracellular accumulation of doxorubicin, the expression of P-glycoprotein, or the expression of the antioxidant glutathione S-transferase-pi mRNA. However, treatment with doxorubicin enhanced the expression of **Fas** on T24 cells. CONCLUSIONS: This study demonstrated that treatment of bladder carcinoma cells with doxorubicin sensitized the cells to lysis by **anti-**

Fas MoAb. The synergistic effect obtained with established doxorubicin-resistant bladder carcinoma cells and freshly isolated bladder carcinoma cells suggests that drug-resistant bladder carcinoma cells can be sensitized by doxorubicin to **Fas**- and **Fas** ligant-mediated cytotoxicity by lymphocytes. Furthermore, the sensitization required low concentrations of doxorubicin, thus supporting the in vivo application of a. . .

CT . . .

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L10 ANSWER 1 OF 6 MEDLINE DUPLICATE 1
AN 2002373026 MEDLINE
DN 22108994 PubMed ID: 12114277
TI Antiproliferative-antiinflammatory effects of **methotrexate** and sex hormones on cultured differentiating myeloid monocytic cells (THP-1).
AU Cutolo Maurizio; Sulli Alberto; Craviotto Chiara; Felli Lamberto; Pizzorni Carmen; Seriolo Bruno; Villaggio Barbara
CS Laboratory and Division of Rheumatology, Department of Internal Medicine and Medical Specialities, University of Genova, Genova, Italy.. mcutolo@unige.it
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (2002 Jun) 966 232-7. Journal code: 7506858. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200208
ED Entered STN: 20020717
Last Updated on STN: 20020816
Entered Medline: 20020815
TI Antiproliferative-antiinflammatory effects of **methotrexate** and sex hormones on cultured differentiating myeloid monocytic cells (THP-1).
AB **Methotrexate** (MTX) is believed to exert both antiproliferative and antiinflammatory effects in a dose-related manner in a majority of rheumatoid arthritis. . . at 72 h versus E2-treated cells; 58% at 96 h versus E2-treated cells; and 41% versus controls, respectively. Bax and **Fas** CD95 expression was found increased in T-treated cells: 14% T at 48 h vs. E(2)-treated cells and controls; 45% T. . . 96 h versus E2-treated cells and 37% versus controls for Bax: 33%, 41%, and 42% T versus E2-treated cells for **Fas**. Moreover, a significant decrease of IL-12 levels in T/MTX treated cells was found at any time when compared to E2-treated cells. In summary, the association of testosterone and MTX compared to MTX alone suggests possible **synergistic** actions. Therefore, the enhancing antiinflammatory effects exerted by androgens might represent a further explanation for the reduced frequency of inflammatory. . .
CT Check Tags: Comparative Study; Human
*Anti-Inflammatory Agents, Non-Steroidal: PD, pharmacology
Antigens, CD95: BI, biosynthesis
Antigens, CD95: GE, genetics
Apoptosis: DE, drug effects
Cell Differentiation: DE, drug effects
Cell Division: DE, drug effects
Drug Synergism
*Estradiol: PD, pharmacology
Gene Expression Regulation, Leukemic: DE, drug effects
*Growth Inhibitors: PD, pharmacology
Interferon Type II: PD, pharmacology
***Methotrexate: PD, pharmacology**
*Monocytes: DE, drug effects
Monocytes: PA, pathology

Neoplasm Proteins: BI, biosynthesis
Neoplasm Proteins: GE, genetics
Proto-Oncogene Proteins: BI,. . .
RN 50-28-2 (Estradiol); 57-85-2 (Testosterone); **59-05-2 (Methotrexate)**; 82115-62-6 (Interferon Type II)

L10 ANSWER 2 OF 6 MEDLINE DUPLICATE 2
AN 1999137510 MEDLINE
DN 99137510 PubMed ID: 9973225
TI Chemotherapy augments TRAIL-induced **apoptosis** in breast cell lines.
AU Keane M M; Ettenberg S A; Nau M M; Russell E K; Lipkowitz S
CS National Cancer Institute, Division of Clinical Sciences, Medicine Branch, National Naval Medical Center, Bethesda, Maryland 20889-5105, USA.
SO CANCER RESEARCH, (1999 Feb 1) 59 (3) 734-41.
Journal code: 2984705R. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199902
ED Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990225
TI Chemotherapy augments TRAIL-induced **apoptosis** in breast cell lines.
AB . . . was investigated in normal and malignant breast epithelial cells. Glutathione-S-transferase (GST)-TRAIL extracellular domain fusion proteins were produced to analyze TRAIL-induced **apoptosis**. Only GST-TRAIL constructs containing regions homologous to the **Fas** self-association and ligand binding domains could induce **apoptosis** . GST-TRAIL induced significant (>90%) **apoptosis** in just one of eight normal and one of eight malignant breast cell lines. All other lines were relatively resistant to TRAIL-induced **apoptosis**. Activating TRAIL receptors DR4 and DR5 were expressed in all normal and malignant breast cell lines. The inhibitory receptor TRID. . . and two of seven malignant breast cell lines. DR4, DR5, or TRID expression did not correlate with sensitivity to TRAIL-induced **apoptosis**. Incubation of cell lines with doxorubicin or 5-fluorouracil significantly augmented TRAIL-induced **apoptosis** in most breast cell lines. By fractional inhibition analysis, the toxicity of the combination of TRAIL and doxorubicin or 5-fluorouracil was **synergistic** compared with either agent alone. In contrast, melphalan and paclitaxel augmented TRAIL-induced **apoptosis** in few cell lines, and **methotrexate** did not augment it in any cell line. Augmentation of TRAIL-induced **apoptosis** by doxorubicin or 5-fluorouracil was mediated through caspase activation. This was evidenced by the fact that chemotherapy agents that **synergized** with TRAIL (e.g., doxorubicin) themselves caused cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP), and their toxicity was blocked by the. . . PARP cleavage, and the combined toxicity also was inhibited by ZVAD-fmk. In contrast, chemotherapy agents that did not augment TRAIL-induced **apoptosis** (e.g., **methotrexate**) caused minimal caspase-3 and PARP cleavage by themselves, and their toxicity was not inhibited by ZVAD-fmk. These drugs also did. . . not increase caspase-3 or PARP cleavage when combined with TRAIL. In summary, few breast cell lines are sensitive to TRAIL-induced **apoptosis**, and no difference in sensitivity is found between normal and malignant cell lines. Treatment with chemotherapy provides an approach to sensitize breast cancer cells to TRAIL-induced **apoptosis**.
CT Check Tags: Human

Antineoplastic Agents: AD, administration & dosage
*Antineoplastic Combined Chemotherapy Protocols: PD, pharmacology
***Apoptosis: DE, drug effects**
***Apoptosis: PH, physiology**
*Breast Neoplasms: DT, drug therapy
*Breast Neoplasms: PA, pathology
Caspases: ME, metabolism
Doxorubicin: AD, administration & dosage
Doxorubicin:. . .

CN 0 (Antineoplastic Agents); 0 (Antineoplastic Combined Chemotherapy Protocols); 0 (Membrane Glycoproteins); 0 (Recombinant Fusion Proteins); 0 (TNF-related **apoptosis**-inducing ligand); 0 (Tumor Necrosis Factor); EC 2.5.1.18 (Glutathione Transferase); EC 3.4.22.- (CPP32 protein); EC 3.4.22.- (Caspases)

L10 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 3
AN 1999:229179 SCISEARCH
GA The Genuine Article (R) Number: 177BC
TI p53-mediated up-regulation of CD95 is not involved in genotoxic drug-induced **apoptosis** of human breast tumor cells
AU RuizRuiz M D; LopezRivas A (Reprint)
CS CSIC, INST PARASITOL & BIOMED, CALLE VENTANILLA 11, GRANADA 18001, SPAIN (Reprint); CSIC, INST PARASITOL & BIOMED, GRANADA 18001, SPAIN
CYA SPAIN
SO CELL DEATH AND DIFFERENTIATION, (MAR 1999) Vol. 6, No. 3, pp. 271-280. Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND.
ISSN: 1350-9047.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 54
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
TI p53-mediated up-regulation of CD95 is not involved in genotoxic drug-induced **apoptosis** of human breast tumor cells
AB Induction of CD95 (**Fas**/APO-1) and CD95 ligand during chemotherapeutic treatment may contribute to the death by **apoptosis** of some tumor cells, In this study, we have analyzed the role of the CD95 system in genotoxic drug-induced death of human breast tumor cells, Incubation of the breast tumor cell lines MCF-7 and EVSA-T with doxorubicin or **methotrexate** caused **apoptosis** after 48 h of treatment, These drugs induced a marked increase in the level of CD95 mRNA and protein in. . . drugs. Furthermore, the genotoxic drugs did not induce the expression of CD95 ligand in MCF-7 cells at doses that caused **apoptosis** in these breast tumor cells. Moreover, drug-induced **apoptosis** of breast tumor cells was not prevented in the presence of either a CD95 antagonistic antibody or a CD95 ligand blocking antibody. We also observed a strong **synergism** between lower doses of DNA-damaging drugs and CD95 agonistic antibody in the induction of **apoptosis** in MCF-7 cells. In summary, our data indicate that drug-induced **apoptosis** of breast tumor cells occurs by a CD95/CD95L-independent mechanism although by elevating the tumor suppressor proteins p53 and CD95, genotoxic drugs may sensitize breast tumor cells to CD95-mediated **apoptosis**.
ST Author Keywords: breast; tumor; **apoptosis**; CD95; CD95L; p53; genotoxic drugs
STP KeyWords Plus (R): APO-1/**FAS** RECEPTOR/LIGAND SYSTEM; TEMPERATURE-SENSITIVE MUTANT; **FAS**-MEDIATED **APOPTOSIS**; MOLECULAR-CLONING; SURFACE ANTIGEN; CARCINOMA CELLS; CANCER PATIENTS; HEPATOMA-CELLS; P53; EXPRESSION

L10 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 4
AN 1998:572926 SCISEARCH
GA The Genuine Article (R) Number: 102MM
TI Sensitization of human bladder cancer cells to **Fas**-mediated cytotoxicity by cis-diamminedichloroplatinum (II)
AU Mizutani Y (Reprint); Yoshida O; Bonavida B
CS KYOTO UNIV, FAC MED, DEPT UROL, SAKYO KU, 54 SHOGOIN KAWAHARA CHO, KYOTO 606, JAPAN (Reprint); UNIV CALIF LOS ANGELES, SCH MED, DEPT MICROBIOL & IMMUNOL, LOS ANGELES, CA 90024
CYA JAPAN; USA
SO JOURNAL OF UROLOGY, (AUG 1998) Vol. 160, No. 2, pp. 561-570.
Publisher: WILLIAMS & WILKINS, 351 WEST CAMDEN ST, BALTIMORE, MD 21201-2436.
ISSN: 0022-5347.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 52
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
TI Sensitization of human bladder cancer cells to **Fas**-mediated cytotoxicity by cis-diamminedichloroplatinum (II)
AB . . . to anticancer chemotherapeutic drugs is a major problem. Several immunotherapeutic approaches have been developed to treat drug-resistant tumor cells. The **Fas** antigen (**Fas**)-**Fas** ligand pathway is involved in cytotoxic T lymphocyte and natural killer cell-mediated cytotoxicity. Like the **Fas** ligand, anti-**Fas** monoclonal antibody (mAb) induces **apoptosis** in tumor cells expressing **Fas**. Several anticancer drugs also mediate **apoptosis** and may share with **Fas** common intracellular pathways leading to cell killing. We reasoned that treatment of drug-resistant cancer cells with a combination of anti-**Fas** mAb and drugs might overcome their resistance. This study has investigated whether anticancer drugs **synergize** with anti-**Fas** mAb in cytotoxicity against bladder cancer cells.

Materials and Methods: Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. **Synergy** was assessed by isobolographic analysis.

Results: Treatment of the T24 human bladder cancer cell line with anti-**Fas** mAb in combination with 5-fluorouracil, mitomycin C or **methotrexate** did not overcome resistance to these agents. However, treatment of T24 tumor cells with a combination of anti-**Fas** mAb and cis-diamminedichloroplatinum (II) (CDDP) resulted in a **synergistic** cytotoxic effect. In addition, the CDDP-resistant T24 line (T24/CDDP) was sensitive to treatment with a combination of anti-**Fas** mAb and CDDP. **Synergy** by combination of anti-**Fas** mAb and CDDP was also achieved in three other bladder cancer lines and four freshly derived human bladder cancer cells. The combination of anti-**Fas** mAb and carboplatin also resulted in a **synergistic** cytotoxic effect on T24 cells; however, the combination of anti-**Fas** mAb and trans-diamminedichloroplatinum (II) resulted in an additive cytotoxic effect. Treatment with CDDP enhanced the expression of **Fas** on T24 cells. The **synergy** achieved in cytotoxicity with anti-Pas mAb and CDDP was also achieved in **apoptosis**. Incubation of T24 cells with anti-Pas mAb increased the intracellular accumulation of CDDP. Treatment of freshly isolated bladder cancer cells. . . . enhanced their susceptibility to lysis by autologous lymphocytes.

Conclusions: This study demonstrates that combination treatment of bladder cancer cells with anti-**Fas** mAb and CDDP overcomes their resistance. **Synergy** was achieved with established CDDP-resistant bladder cancer cells and freshly isolated bladder cancer cells. In

addition, the sensitization required low. . .
ST Author Keywords: **Fas**; CDDP; bladder cancer; **synergy**; **apoptosis**
STP KeyWords Plus (R): TUMOR-NECROSIS-FACTOR; PERIPHERAL-BLOOD LYMPHOCYTES; L1210 LEUKEMIA-CELLS; OVERCOMING TNF-ALPHA; RNA DOWN-REGULATION; CARCINOMA-CELLS; MESSENGER-RNA; ANTI-**FAS**; BUTHIONINE SULFOXIMINE; COMBINATION TREATMENT

L10 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 5
AN 1998:478757 SCISEARCH
GA The Genuine Article (R) Number: ZU799
TI Chemotherapeutic drug-induced **apoptosis** in human leukaemic cells is independent of the **Fas** (APO-1/CD95) receptor/ligand system
AU McGahon A J; Pereira A P C; Daly L; Cotter T G (Reprint)
CS NATL UNIV IRELAND UNIV COLL CORK, DEPT BIOCHEM, TUMOUR BIOL LAB, PROSPECT ROW, CORK, IRELAND (Reprint); NATL UNIV IRELAND UNIV COLL CORK, DEPT BIOCHEM, TUMOUR BIOL LAB, CORK, IRELAND
CYA IRELAND
SO BRITISH JOURNAL OF HAEMATOLOGY, (JUN 1998) Vol. 101, No. 3, pp. 539-547. Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0007-1048.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
TI Chemotherapeutic drug-induced **apoptosis** in human leukaemic cells is independent of the **Fas** (APO-1/CD95) receptor/ligand system
AB The potential role of the **Fas** (CD95/APO-1) receptor/ligand system in chemotherapeutic drug-induced **apoptosis** was examined in a number of human leukaemic cell lines. Flow cytometric profiles of doxorubicin-treated HL-60, K562, U937 and Jurkat cells failed to show any significant increase in **Fas** or **Fas** ligand expression over 24 h, despite the induction of significant levels of **apoptosis** in these cells, Although preincubation of human leukaemic cells with a neutralizing anti-**Fas** IgG antibody blocked anti-**Fas** IgM-induced **apoptosis**, this strategy failed to inhibit chemotherapeutic drug-induced **apoptosis**, To determine whether recruitment of the **Fas**/**Fas** ligand complex during drug-induced **apoptosis** was a cell-specific event we utilized the CEM cell line. Doxorubicin treatment of CEM cells over 24 h failed to show any up-regulation in **Fas** or **Fas** ligand protein levels as detected by now cytometry. Furthermore, neutralizing anti-**Fas** IgG Ab failed to inhibit chemotherapeutic drug-induced **apoptosis** in CEM cells. The present studies do, however, demonstrate a role for anti-**Fas** IgM Ab in producing a cytotoxic **synergistic** effect when used in combination with chemotherapeutic drugs. Low-dose anti-**Fas** IgM treatment in combination with doxorubicin, **methotrexate**, camptothecin and etoposide produced an augmented cytoxicity in CEM cells. Taken together these observations demonstrate that although recruitment of the **Fas**/APO-1/CD95 receptor/ligand system is not a necessary requirement for chemotherapeutic drug-induced **apoptosis**, combination of anti-**Fas** IgM and drug treatment produces a **synergistic** cytotoxic effect which may prove useful in the treatment of human leukaemias.
ST Author Keywords: **apoptosis**; leukaemia; **Fas**; cytotoxic

L10 ANSWER 6 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 6
AN 97:218689 SCISEARCH

GA The Genuine Article (R) Number: WM576
TI Doxorubicin sensitizes human bladder carcinoma cells to **Fas** -mediated cytotoxicity
AU Mizutani Y; Okada Y; Yoshida O (Reprint); Fukumoto M; Bonavida B
CS KYOTO UNIV, FAC MED, DEPT UROL, SAKYO KU, 54 SHOGOIN KAWAHARA CHO, KYOTO 606, JAPAN (Reprint); KYOTO UNIV, FAC MED, DEPT UROL, SAKYO KU, KYOTO 606, JAPAN; KYOTO UNIV, FAC MED, DEPT PATHOL 1, KYOTO, JAPAN; UNIV CALIF LOS ANGELES, SCH MED, DEPT MICROBIOL & IMMUNOL, LOS ANGELES, CA 90024
CYA JAPAN; USA
SO CANCER, (15 MAR 1997) Vol. 79, No. 6, pp. 1180-1189.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC 605 THIRD AVE, NEW YORK, NY 10158-0012.
ISSN: 0008-543X.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 44
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
TI Doxorubicin sensitizes human bladder carcinoma cells to **Fas** -mediated cytotoxicity
AB . . . anticancer chemotherapeutic agents remains a major problem. Hence, several immunotherapeutic approaches have been developed to treat the drug-resistant cancer cells. **Fas** antigen (**Fas**) and **Fas** Ligand participate in cytotoxicity mediated by T lymphocytes and natural killer cells. Like **Fas** ligand, anti-**Fas** monoclonal antibody (MoAb) induces **apoptosis** of the cells expressing **Fas**. This study examined whether bladder carcinoma cells are sensitive to cytotoxicity mediated hp anti-**Fas** MoAb and whether anticancer agents **synergize** with anti-**Fas** MoAb in cytotoxicity. METHODS. Cytotoxicity was determined by a 1-day microculture tetrazolium dye assay. **Synergy** was assessed by isobolographic analysis.

RESULTS. The T24 human bladder carcinoma cell line constitutively expressed the **Fas** on the cell surface; however, T24 Line was resistant to anti-**Fas** MoAb. Treatment of T24 cells with anti-**Fas** MoAb in combination with mitomycin C, **methotrexate**, or S-fluorouracil did not overcome their resistance to these agents, However, treatment of T24 cells with a combination of anti-**Fas** MoAb and doxorubicin resulted in a **synergistic** cytotoxic effect. In addition, the doxorubicin-resistant T24 cells were sensitive to treatment with a combination of anti-**Fas** MoAb and doxorubicin. **Synergy** was also achieved in three other bladder carcinoma cell lines and four freshly derived human bladder carcinoma cells. Treatment with anti-**Fas** MoAb in combination with epirubicin or pirarubicin also resulted in a **synergistic** cytotoxic effect on T24 cells. The mechanisms of **synergy** were examined. Anti-**Fas** MoAb did not affect the intracellular accumulation of doxorubicin, the expression of P-glycoprotein, or the expression of the antioxidant glutathione S-transferase-pi mRNA. However, treatment with doxorubicin enhanced the expression of **Fas** on T24 cells.

CONCLUSIONS. This study demonstrated that treatment of bladder carcinoma cells with doxorubicin sensitized the cells to lysis by anti-**Fas** MoAb. The **synergistic** effect obtained with established doxorubicin-resistant bladder carcinoma cells and freshly isolated bladder carcinoma cells suggests that drug-resistant bladder carcinoma cells can be sensitized by doxorubicin to **Fas**- and **Fas** ligant-mediated cytotoxicity by lymphocytes. Furthermore, the sensitization required low concentrations of doxorubicin, thus supporting the in vivo application of a. . .
ST Author Keywords: **Fas**; doxorubicin; bladder carcinoma; **synergy**; **apoptosis**

STP KeyWords Plus (R): TUMOR-NECROSIS-FACTOR; RESISTANT P388 LEUKEMIA; PERIPHERAL-BLOOD LYMPHOCYTES; TOPOISOMERASE-II ACTIVITY; RNA DOWN-REGULATION; ANTI-**FAS**; COMBINATION TREATMENT; RECEPTOR SUPERFAMILY; MONOCLONAL-ANTIBODY; MOLECULAR-CLONING

L15 ANSWER 1 OF 4 MEDLINE
AN 2002230970 IN-PROCESS
DN 21954176 PubMed ID: 11957195
TI Chronic neutropenia associated with **autoimmune** disease.
AU Starkebaum Gordon
CS Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.
SO SEMINARS IN HEMATOLOGY, (2002 Apr) 39 (2) 121-7. Ref: 58
Journal code: 0404514. ISSN: 0037-1963.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020424
Last Updated on STN: 20021211
TI Chronic neutropenia associated with **autoimmune** disease.
AB Chronic neutropenia with **autoimmune** diseases is associated mainly with **rheumatoid** arthritis (RA), as Felty's syndrome or large granular lymphocyte (LGL) leukemia, and with systemic lupus erythematosus (SLE). Recent advances have allowed better understanding regarding the mechanism of neutropenia and improved options for treatment. Target antigens for antineutrophil **antibodies** have been identified for both Felty's syndrome and for SLE. The role of soluble **Fas**-ligand (FasL) in inducing **apoptosis** of neutrophils has been clarified for LGL leukemia and increased neutrophil **apoptosis** has been described in neutropenic patients with SLE. The role of immune complexes in affecting neutrophil traffic and function continues to be studied. Treatments of neutropenia have included **methotrexate**, cyclosporine A, and granulocyte colony-stimulating factor (G-CSF) as well as granulocyte-macrophage colony-stimulating factor (GM-CSF). The efficacy of both GM- and. . .

L15 ANSWER 2 OF 4 MEDLINE
AN 1998330458 MEDLINE
DN 98330458 PubMed ID: 9664073
TI Immunosuppressive properties of **methotrexate**: **apoptosis** and clonal deletion of activated peripheral T cells.
AU Genestier L; Paillot R; Fournel S; Ferraro C; Miossec P; Revillard J P
CS Laboratory of Immunology, Institut National de la Sante et de la Recherche Medicale U80 Claude Bernard University, Hopital E. Herriot, 69437 Lyon, France.
SO JOURNAL OF CLINICAL INVESTIGATION, (1998 Jul 15) 102 (2) 322-8.
Journal code: 7802877. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199808
ED Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980820
TI Immunosuppressive properties of **methotrexate**: **apoptosis** and clonal deletion of activated peripheral T cells.
AB The folate antagonist **methotrexate** (MTX) is extensively used in graft-versus-host disease, **rheumatoid** arthritis, and other chronic inflammatory disorders. In addition to its antiinflammatory activity associated with increased release of adenosine, MTX exerts. . . mechanisms of immunosuppressive properties associated with low-dose MTX treatments are still elusive. We report here that MTX (0.1-10 microM)

induces **apoptosis** of in vitro activated T cells from human peripheral blood. PBL exposed to MTX for 8 h, then activated in drug-free medium, underwent **apoptosis**, which was completely abrogated by addition of folinic acid or thymidine. **Apoptosis** of activated T cells did not require interaction between CD95 (**Fas**, APO-1) and its ligand, and adenosine release accounted for only a small part of this MTX activity. **Apoptosis** required progression of activated T cells to the S phase of the cell cycle, as it was prevented by drugs or **antibodies** that interfere with IL-2 synthesis or signaling pathways. MTX achieved clonal deletion of activated T cells in mixed lymphocyte reactions. Finally, in vitro activation of PBL taken from **rheumatoid** arthritis patients after MTX injection resulted in **apoptosis**. Altogether, the data demonstrate that MTX can selectively delete activated peripheral blood T cells by a CD95-independent pathway. This property. . .

CT Check Tags: Human; Support, Non-U.S. Gov't
Adenosine: PD, pharmacology
***Apoptosis**
Arthritis, Rheumatoid: BL, blood
Cell Cycle
Cells, Cultured
*Clonal Deletion: IM, immunology
Culture Media
Folic Acid Antagonists: PD, pharmacology
*Immunosuppressive Agents: PD, pharmacology
Leukocytes, Mononuclear
Lymphocyte Transformation
***Methotrexate: PD, pharmacology**
Mitogens: PD, pharmacology
Phytohemagglutinins: PD, pharmacology
S Phase
T-Lymphocytes: CY, cytology
*T-Lymphocytes: DE, drug effects
T-Lymphocytes: IM, immunology

. . .

RN 58-61-7 (Adenosine); **59-05-2 (Methotrexate)**

L15 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2003 ISI (R)
AN 1999:982911 SCISEARCH
GA The Genuine Article (R) Number: 265NH
TI Inducers of cytochrome P450 2E1 enhance **methotrexate**-induced hepatocytotoxicity
AU Neuman M G (Reprint); Cameron R G; Haber J A; Katz G G; Malkiewicz I M; Shear N H
CS SUNNYBROOK HLTH SCI CTR, DIV CLIN PHARMACOL, E-240, 2075 BAYVIEW AVE, TORONTO, ON M4N 3M5, CANADA (Reprint); UNIV TORONTO, TORONTO HOSP, DEPT PATHOL, TORONTO, ON, CANADA; UNIV TORONTO, DEPT PHARMACOL, TORONTO, ON, CANADA; UNIV TORONTO, DEPT MED, TORONTO, ON, CANADA; UNIV TORONTO, DEPT PATHOL, TORONTO, ON, CANADA; UNIV TORONTO, SUNNYBROOK & WOMENS COLL HLTH SCI CTR, DIV CLIN PHARMACOL, TORONTO, ON, CANADA
CYA CANADA
SO CLINICAL BIOCHEMISTRY, (OCT 1999) Vol. 32, No. 7, pp. 519-536.
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
ISSN: 0009-9120.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 77
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
TI Inducers of cytochrome P450 2E1 enhance **methotrexate**-induced

hepatocytotoxicity

AB Objectives: To study the effect of cytochrome P450 2E1-inducers on **methotrexate** (MTX)-induced cytotoxicity in human hepatocytes, and investigate the role of silymarin in preventing this toxicity.
Design and methods: Cells were exposed to MTX in the presence of either ethanol (EtOH) or acetaminophen (APAP), or either combined with silymarin (S). **Apoptosis** and necrosis were measured by analyzing 6000 cells/sample using transmission electron microscopy, while cytokine release and **apoptosis** were quantitated by ELISA. Cytokine expression was measured by RT-PCR. Gluthatione (GSH) content was determined in cytosolic (c) and mitochondrial. . . 0.5 mmol/L S downregulated TNF a expression and reduced cytokine release. TNF a increased cytotoxicity by 22%, while anti-TNF alpha **antibody** eradicated it. MTX + EtOH depleted 45% mGSH ($p < 0.001$) while S replenished it to 87% ($p < 0.001$),. . . oxidative stress in MTX-exposed cells by increasing TNF alpha and depleting both cGSH and mGSH. This enhances MTX-cytotoxicity and promotes **apoptosis**. Copyright (C) 1999 The Canadian Society of Clinical Chemists.

ST Author Keywords: **methotrexate**; cytochrome P350 2E1; **apoptosis**; necrosis; acetaminophen; ethanol; Hep G2; normal human primary hepatocytes

STP KeyWords Plus (R): TUMOR-NECROSIS-FACTOR; APO-1/**FAS** RECEPTOR/LIGAND SYSTEM; SEVERE ALCOHOLIC HEPATITIS; DRUG-INDUCED **APOPTOSIS**; NATURAL-KILLER-CELLS; IN-VITRO; **RHEUMATOID** -ARTHRITIS; RAT HEPATOCYTES; FACTOR-ALPHA; HEP G2

L15 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:241398 BIOSIS
DN PREV200200241398
TI Immune modulation with low-dose **methotrexate** involves mitochondrial-mediated **apoptosis** of CD8+ lymphocytes.
AU Epling-Burnette, P. K. (1); Al-Dawoodie, Nasrin (1); Bai, Fanqui (1); Loughran, Thomas P. (1)
CS (1) Moffitt Cancer Center, IOP, Uni. S. Florida, Tampa, FL USA
SO Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 510a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DT Conference
LA English
TI Immune modulation with low-dose **methotrexate** involves mitochondrial-mediated **apoptosis** of CD8+ lymphocytes.
AB **Methotrexate** is widely used as a disease-modifying anti-**rheumatic** drug (DMARD) for the treatment of **rheumatoid** arthritis (RA) and other **autoimmune** diseases. It has also been successfully used for the treatment of Large Granular Lymphocyte (LGL) Leukemia which is characterized by the amplification of CD8+ T lymphocytes with an activated phenotype. Although millions of patients are presently taking low-dose **methotrexate** as immune-modulating therapy, the mechanism of action is poorly understood. We found that doses between 8 nM to 1000 nM selectively induced **apoptosis** (phosphatidylserine externalization) of the CD8+ lymphocyte population from peripheral blood (PBMC) after activation with PHA and IL-2 and in an. . . lymphocytic cell line, CEM. Unactivated PBMC and monocytic cell lines (K562 and HL-60) were resistant to these low doses of **methotrexate**. We found that **apoptosis** was reversed by pretreatment with folinic acid but not antagonist **anti-Fas antibody** suggesting that inhibition of dihydrofolate reductase (DHFR) is involved in the induction of **apoptosis** but not ligation of the **Fas** receptor. In addition to externalization of phosphatidylserine (as measured by binding

of Annexin-V FITC and detected by flow cytometry), we. . . by flow cytometry. We also detected the presence of fragmented DNA using a fluorescent tunel assay (Intergen, Purchase, NY). Interestingly, **methotrexate**-induced **apoptosis** was not reduced by pre-treatment of the cells with a cell permeable caspase 3 inhibitor (Ac-DEVD-fmk, Calbiochem). Also, activation of. . . as indicated by caspase 3 cleavage was not evident by Western blot anlaysis, although readily apparant in cells treated with **anti-Fas** agonistic **antibody** used as a positive control. These data provide us with new and exciting information about the commonly used immune-modulating drug, **methotrexate**. Our data suggests that a proliferating population of lymphocytes are responsive to the apoptotic effects of **methotrexate** after mitochondrial disruption and DNA fragmentation independent of caspase 3 activation. It is important to further delineate the effector caspase. . .

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Pharmacology

IT Parts, Structures, & Systems of Organisms
CD8 positive lymphocyte: **apoptosis**, blood and lymphatics, immune system; mitochondria; peripheral blood: blood and lymphatics

IT Chemicals & Biochemicals
Ac-DEVD-fmk: enzyme inhibitor - drug; DNA: fragmentation; **Fas** receptor; IL-2 [interleukin-2]; PHA; caspase 3: cleavage; dihydrofolate reductase: regulation; folinic acid; **methotrexate**: antiarthritic - drug, antineoplastic - drug, enzyme inhibitor - drug, immunologic - drug, immunosuppressant - drug, pharmacodynamics; phosphatidylserine

RN 169592-56-7 (CASPASE 3)
9002-03-3 (DIHYDROFOLATE REDUCTASE)
58-05-9 (FOLINIC ACID)
59-05-2 (**METHOTREXATE**)

WEST Search History

DATE: Tuesday, March 18, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR		
L5	L4 and Serizawa.in.	11	L5
L4	L3 not l2	105	L4
L3	anti-fas with antibody and (autoimmune or auto-immune or rheumat$)	229	L3
	DB=USPT; PLUR=YES; OP=OR		
L2	anti-fas with antibody and (autoimmune or auto-immune or rheumat$)	124	L2
L1	6153615.pn. and methotrex$	1	L1

END OF SEARCH HISTORY